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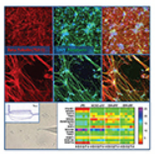

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REVIEW

The role of DNA methylation in thermogenic adipose biology

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ABSTRACT

The two types of thermogenic fat cells, beige and brown adipocytes, play a significant role in regulating energy homeostasis. Their development and thermogenesis are tightly regulated by dynamic epigenetic mechanisms, which could potentially be targeted to treat metabolic disorders such as obesity. However, we are just beginning to catalog and understand these dynamic changes. In this review, we will discuss the current understanding of the role of DNA (de) methylation events in beige and brown adipose biology in order to highlight the holes in our knowledge and to point the way forward for future studies.

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Introduction

At least three types of adipose tissue exists in mammals: white, beige, and brown [1,2]. White adipocytes store excess energy as triglycerides and release them as needed, whereas brown adipocytes burn that energy to create heat [1,2]. Beige adipocytes sit between the two phenotypes, seemingly alternating between storing energy and burning it [1,2]. In rodents, classical brown adipose tissue exists in defined anatomical depots, such as the interscapular regions [1,2]. Concordantly, human studies detect thermogenic adipocytes around the neck, clavicle and spinal cord [3–7] that burn glucose and fatty acids [8,9]. Beige adipocytes aren't in depots, but instead, are interspersed within white adipose tissue (WAT) [10].

For thermogenesis, both beige and brown adipocytes have abundant mitochondria to oxidize fatty acids, thus generating heat via uncoupling protein 1 (UCP1)-dependent and independent mechanisms [10,11]. Beige adipocytes biogenesis in WAT is induced by various environmental cues, including cold exposure, exercise, and PPARγ agonist [12,13], in a process called 'browning' or 'beiging'[14]. Conversely, they undergo 'whitening' in response to thermoneutrality, impaired β-adrenergic signaling, lipase deficiency, and other cues [15]. Brown adipocytes also exhibit a certain degree of flexibility in their thermogenic gene program and morphology [16,17]. Because chronic cold acclimatization in humans leads to increased adipose thermogenic activity, which leads to increased energy expenditure [18,19], beige and brown adipocytes are an attractive therapeutic target for obesity and related metabolic diseases [19–21].

Studies strongly suggest that DNA (de)methylation plays a critical role in thermogenic adipose development and gene regulation. The reversible nature of epigenetic changes raises hope for therapeutic interventions that can reverse deleterious epigenetic programing as a means to prevent or treat relevant metabolic disorders. However, a deeper understanding is needed before medical therapies can be developed to target the epigenome. Here, we will discuss the current understanding of the role of DNA (de)methylation events in beige and brown adipose biology, with a focus on their development and gene regulation.

DNA (De)methylation in brown adipogenesis

PR domain–containing 16 protein (PRDM16) is a key developmental transcriptional regulator that commits progenitors to the brown adipogenic lineage and maintains brown adipocyte identity [22]. *Prdm16* is enriched with CpG sites around its transcription start site, and hypomethylation at three specific regions of its promoter, likely mediated by the TET proteins, leads to increased *Prdm16*

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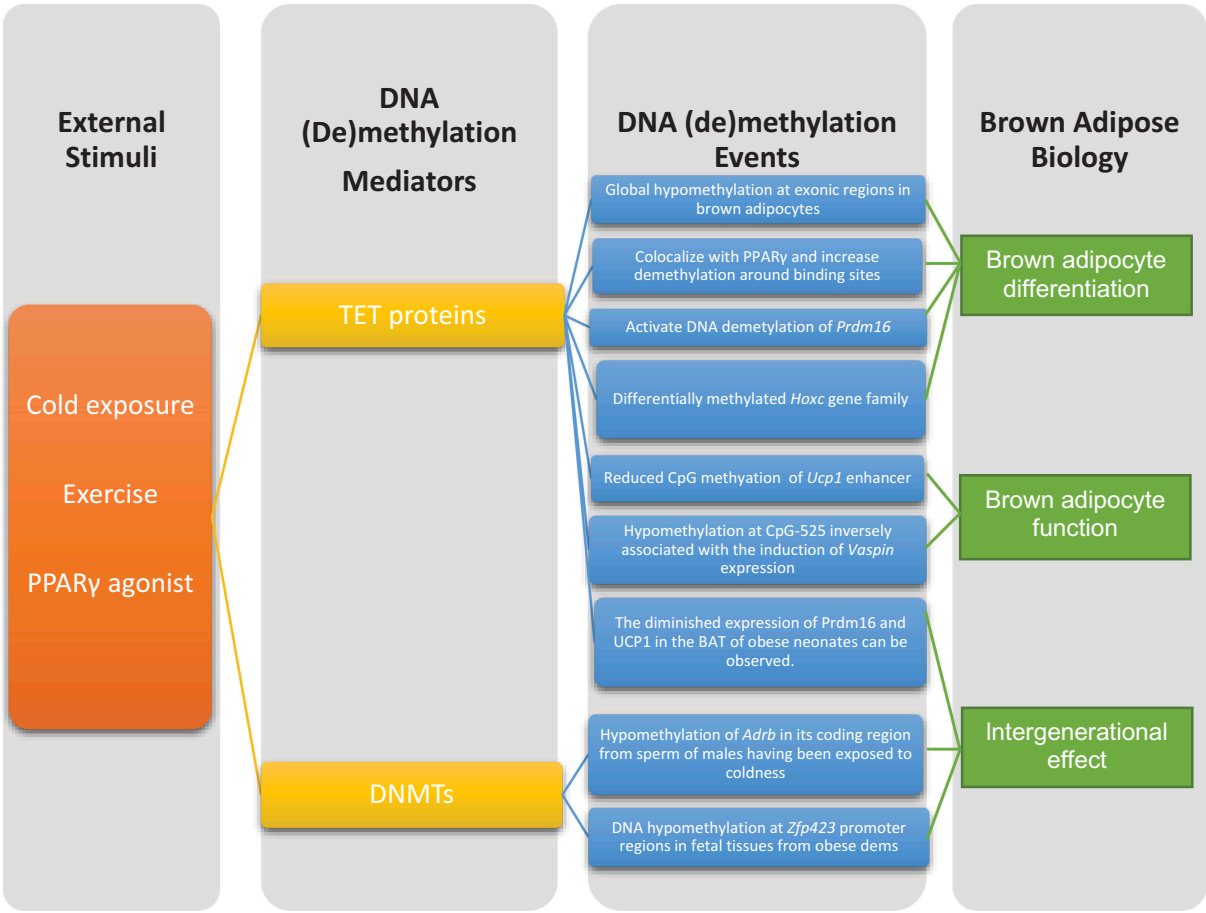

Figure 1. Summary of external stimuli and DNA (de)methylation machinery and events that affect thermogenic adipose biology.

expression during brown adipogenesis [23,24]. Moreover, reducing the level of α-ketoglutarate (αKG), a co-factor for the TET enzymes, leads to reduced demethylation of *Prdm16* and impaired brown adipocyte development and function in mutant mice carrying loss-of-function of AMPKa1 [23].

To find loci important for brown adipose development, two independent DNA methylome studies were conducted to identify differentially methylated regions between white and brown adipocytes. The first study differentiated stromal vascular cells into inguinal and brown adipose cells *in vitro* [25]. The authors found that white adipogenesis has more hypermethylation overall than brown adipogenesis, and it is located mostly at intronic and intergenic regions. On the other hand, brown adipocytes have hypomethylated exonic regions that are significantly enriched for genes involved in brown fat functions such as the mitochondrial respiratory chain and fatty acid oxidation [25]. Notably, several Hox transcription factors are differentially methylated, some of which are linked to adipogenesis and diabetes [26,27]. For example, *Hoxc9* is a well-established adipocyte marker [28], and *Hoxc9* and *Hoxc10* promoter methylation is inversely correlated with their gene expression in brown adipose tissue.

The second global study compared the DNA methylation profile of primary white vs. brown pre-adipocytes, among other cell types. Here, authors concluded that the DNA methylome is greatly similar between white and brown adipocytes [29]. However, there are multiple variables that could account for the discrepancy, including the cell types used (*in vitro* differentiated vs. primary cells), differential genome coverages due to the profiling method (reduced representation bisulfite sequencing vs. restriction hallmark genomic scanning), and the number of comparative analyses (two cell types vs. multiple comparisons between multiple cell types). Future genome-wide studies using whole-genome bisulfite sequencing

are needed to compare, at base-pair resolution, the DNA methylation events between white and brown adipogenesis.

There are likely many regions involved in thermogenic adipogenesis that are controlled epigenetically, as global inhibition of (de)methylation greatly impacts general adipogenesis. The expression of TETs, the mediators of DNA demethylation, is upregulated in tissue culture models of both white and brown adipogenesis [23,30]. In addition, TET1 appears to use a physical interaction with a nuclear receptor (PPARγ) to target adipose genes during differentiation [31,32]. This results in demethylation and H3K4me1/H3K27ac around PPARγ binding sites in 3T3-L1 adipocytes [31–33]. Interestingly, in mature adipocytes, TET2 facilitates the transcriptional activity of PPARγ and the insulin-sensitizing efficacy of PPARγ agonist by sustaining PPARγ DNA binding at certain target loci [34]. These studies employed non-brown/beige adipocyte cell lines, yet it is likely that the TETs play additional roles in thermogenic adipocytes – outside of their effect on *Prdm16*. These roles require further studies using beige and brown adipocyte models.

Opposing the TETs are DNMTs, the mediators of DNA methylation. No specific role in brown adipogenesis has been found for DNMTs; however, they are likely important, as they have huge effects on general adipogenesis. Pharmacological and genetic inhibition of DNMTs appears to have biphasic impact on adipogenesis. Administering DNMT inhibitor prior to or during the early stages of adipogenesis enhanced adipogenesis [35–38]. This holds true in multiple tissue culture models including multipotent C3H10T1/2, ST2 cells, and pre-white adipocytes 3T3-L1[36–]38]. However, the opposite effect is observed when the inhibitor is added at a later stage of differentiation [39]. Knockdown of *Dnmt1* and *Dnmt3a* during clonal expansion or early adipogenesis (day 0–2) impairs 3T3-L1 adipogenesis [39–41] but promotes lipid accumulation when knocked down on day 5 [39].

The specific effect of these DNMTs may depend on their expression pattern. *Dnmt1* expression is transiently increased during the mitotic clonal expansion phase [42], which is critical for *in vitro* adipogenesis [43], and reduced in later stages of differentiation [42]. By contrast, *Dnmt3a* expression is increased during later stages of adipogenesis, while *Dnmt3b* expression remains low and relatively stable during differentiation. Together, these studies suggest that DNA methylation, along with DNMT1 and 3a, has complex roles in adipogenesis depending on the stage of adipose conversion. However, another group reported that DNMT1 is anti-adipogenic even during early phases by showing that DNMT1 is necessary for maintaining DNA methylation and repressive H3K9 histone methylation at key adipogenic genes, such as *Pparg*, during clonal expansion [42]. Such a discrepancy might be due to the knockdown efficiency of *Dnmt1* or tissue culture variables between the two laboratory environments. While it is likely that DNMTs play a role in brown adipogenesis, future studies are necessary to reveal their exact functional role.

DNA methylation in brown adipocyte gene regulation

UCP1 is important for adipocyte thermogenesis, as it uncouples the respiratory chain, allowing for fast substrate oxidation with a low rate of ATP production. Brown adipocyte-specific *Ucp1* expression is associated with reduced CpG methylation at the *Ucp1* enhancer and can be further reduced by DNMT inhibitor in brown adipocyte HIB1B cells [44]. Moreover, cold adaptation causes DNA hypomethylation at the CpG sites within two of the cyclic AMP response elements in the *Ucp1* promoter [44]. Consistent with this, under cold conditions, the *Ucp1* locus is more enriched in the active histone mark (H3K4me3) in brown adipose tissue (BAT), whereas the repressive mark (H3K9me2) is enriched in white adipose tissue (WAT) [44].

Peroxisome proliferator–activated receptor gamma coactivator 1 alpha (PGC-1α) is required for the cold-inducible expression of *Ucp1* [45]. PGC-1α is a transcriptional co-activator that regulates genes involved in energy metabolism, and its methylation changes in the context of insulin resistance and exercise in tissues like skeletal muscle [46,47]. However, whether temperature changes cause changes in *Ppargc1a* methylation in beige and brown adipocytes remains to be uncovered.

Another study suggests that DNA methylation is involved in the brown adipocyte–specific expression of Vaspin (visceral adipose tissue–derived serine

protease inhibitor; SERPINA12). Vaspin is an adipokine suggested to be anti-diabetic and anti-obesogenic [48] because it inhibits hepatic gluconeogenesis and improves insulin signal transduction [49–51]. A microarray study found that *Vaspin* level is strongly upregulated in BAT after cold exposure, and another study found that *Vaspin* promoter regions in BAT are more hypomethylated than white adipose depots in mice fed with normal chow. Moreover, acute cold exposure further decreases methylation levels, notably at one CpG site (CpG-525). Supporting the *in vivo* findings, *in vitro* experiments demonstrate that vaspin mRNA expression is markedly upregulated after treating BAT pre-adipocytes with the DNMT inhibitor 5-aza-2′-deoxycytidine for 48h prior to differentiation. Future studies are warranted to address the causal role of DNA methylation in brown adipose–specific gene regulation in response to various physiological stimuli.

DNA methylation in intergenerational and transgenerational brown adipocyte function

Intergenerational effects occur when the parental environment (F0) directly affects their germ cells or developing fetus (F1). A true transgenerational effect can only be proven if the effect of exposure is transmitted to the F2 (when parental exposure occurred before conception) or F3 (when maternal exposure occurred during pregnancy) [52]. Accumulating evidence supports that DNA methylation plays an important role in the heritability of obesity and other metabolic disorders. A classic example is the *Agouti* (A^{vy}) mouse model. Ectopic expression of the *Agouti* gene during development, due to hypomethylation of the cryptic promoter, results in agouti fur, as well as adult-onset obesity, diabetes, and tumorigenesis [53]. The tendency for obesity is exacerbated when the A^{vy} allele comes from an obese A^{vy} mother [54], and this intergenerational effect is partially reversed by supplementing with methyl-donors, which promote DNA hypermethylation [54]. In addition, in humans, several genes important for development and metabolism, such as *IGF2* and *LEP*, are differentially methylated in newborns that were prenatally exposed to famine and overnutrition [55,56].

A recent study revealed the link between DNA methylation and the intergenerational impact of environmental exposure on brown adipose activity [57]. Notably, cold exposure in males, but not females, prior to conception results in increased cold tolerance and improved whole-body metabolism in male offspring in association with enhanced expression of UCP1 in BAT [57]. This intergenerational transmission is associated with altered DNA methylation at multiple genomic *loci* within the sperm – most prominently in the gene body of *Adrb3*, which encodes a protein that mediates β-adrenergic stimulation in BAT, was hypomethylated in sperm [57]. This led to increased expression of *Adrb3* in inguinal, epididymal, and BAT of the cold exposed offsprings [57]. This study supports the possibility that DNA methylation underlies the epigenetic basis of the sexually dimorphic inheritance of prenatal cold exposure.

Another intriguing study showed that neonates born to obese wild-type mice have reduced brown adipose activity [23], a finding that is correlated with obesity [19,58]. These mice have reduced *Prdm16* expression, in association with a reduced α-KG level, due to DNA hypermethylation at *Prdm16* [23]. As a result, *Ucp1* expression is reduced in these offspring, impairing the ability to maintain body temperature in response to cold [23]. Interestingly, administering AMPK agonists, like metformin and AICAR, which increase α-KG level by activating AMPK-dependent signaling pathways after birth, rescues the inherited obesity-induced suppression of brown adipogenesis and adaptive thermogenesis in offspring [23]. Consistent with this mouse study, a human study reported that maternal obesity increases DNA methylation in the *Prdm16* promoter in the placenta at birth [59].

Zinc-finger protein 423 (Zfp423) is a preadipocyte commitment factor during fetal development [60]. It maintains white adipocyte identity by suppressing EBF2/PPARγ-dependent *Prdm16* induction [61]. Maternal obesity led to DNA hypomethylation at *Zfp423* and the increased gene expression in whole fetal tissues from embryos, which results in increased adipogenesis in the offspring at weaning and increased susceptibility to obesity later in life [62]. Similar to this study, a global profiling study detected DNA hypomethylation at *Zfp423* promoter regions in the adipose tissue from obese dams compared to controls [63].

Another protein involved in transgenerational regulation is PPARγ, which is the master transcription factor for both white and brown adipogenesis and is involved in brown adipocyte development and thermogenic gene regulation [64,65]. Offspring with obese mothers have persistently lower PPARγ expression due to higher epigenetic repression, such as DNA hypermethylation and fewer active histone markers, at the *Pparg* promoter region [66]. Follow-up studies are necessary to address whether these DNA methylation and transcriptional changes impact brown/beige adipose development in neonates and later in life. In addition, more studies are needed to determine whether this altered thermogenic fat biology and its associated metabolic effects are truly transgenerational and whether DNA (de) methylation is involved in that process.

Conclusions and future perspectives

Studies strongly suggest that DNA (de)methylation plays a critical role in brown adipose development and thermogenic gene regulation (summarized in Figure 1). However, a deeper understanding is needed before we can target them in the treatment of obesity and related disorders. For example, what is the role of DNA (de)methylation in regulating 'browning' and 'whitening' and how dynamically are they regulated in response to various external cues? Also, little is known about the putative interaction between DNA (de)methylation and other epigenetic mechanisms in governing thermogenic brown/beige adipogenesis and plasticity. Furthermore, it's crucial to investigate whether the machinery is functionally implicated in thermogenic brown/beige adipose development and function in humans. In conclusion, elucidating the role of DNA (de)methylation in brown and beige adipose biology will shed light on effective therapeutic interventions for obesity and obesity-related human diseases.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Author Contributions

SK and HX co-wrote manuscript and HX did artwork.

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